The effects of low-density lipoprotein and cholesterol on acyl-coenzyme A:cholesterol acyltransferase activity in membranes from cultured human fibroblasts

Sean J. P. GAVIGAN and Brian L. KNIGHT

Medical Research Council Lipid Metabolism Unit, Hammersmith Hospital, DuCane Road, London W12 0HS, U.K.

(Received 10 May 1983/Accepted 7 July 1983)

Membranes prepared from cultured fibroblasts were assayed for acyl-coenzyme A:cholesterol acyltransferase (ACAT) by a method that relied exclusively on the cholesterol already present on the membranes as the sterol substrate. Changes in membrane ACAT activity during incubation of fibroblasts under a variety of conditions were similar to the changes in the rate of incorporation of oleic acid into cholesteryl esters by the intact cells. The addition of low-density lipoprotein (LDL) to fibroblasts pre-incubated with lipoprotein-deficient serum led to a transient increase in membrane ACAT activity, which reached its peak after 7 h and was related to the receptor-mediated uptake and degradation of the lipoprotein by the cells. However, after incubation of the membranes with a cholesterol-rich donor lipoprotein, which resulted in an equilibration of cholesterol between membranes and donor, each preparation exhibited the same activity. In contrast with these effects of LDL, incubation of the cells with non-esterified cholesterol produced a prolonged increase in ACAT activity and an increase in the activity observed after equilibration. Furthermore, ACAT activity in cells grown with linoleic acid was higher, both before and after the addition of LDL, than that of cells grown in normal medium or with palmitate. The increase in activity produced by LDL was also greater, reflecting the greater rate of degradation of LDL by the cells, and was associated with an increase in the activity observed after equilibration with donor. The results suggest that although fibroblasts can increase the amount of active enzyme on their membranes to accommodate an exceptionally high or prolonged supply of cholesterol, under normal circumstances the increase in membrane ACAT activity produced by LDL can be explained entirely by an increase in the amount of cholesterol in the substrate pool.

The development of an atherosclerotic lesion is associated with an accumulation of cholesteryl esters within cells of the arterial wall (Ross, 1981). The cholesterol is thought to be derived mainly from the uptake and lysosomal hydrolysis of cholesterol-rich lipoproteins (Goldstein & Brown, 1977), and its subsequent esterification is catalysed by ACAT, a membrane-bound enzyme found in the rough endoplasmic reticular fraction of the microsomal pellet (Goodman *et al.*, 1963; Balasubramaniam *et al.*, 1978b). Much of our information about the cellular

uptake of lipoproteins has come from experiments with cultured cells, in particular human fibroblasts (Goldstein & Brown, 1977). However, studies on the formation of cholesteryl esters in these cells have been limited to observing changes in mass or in the net incorporation of radioactive fatty acids from the medium (Goldstein *et al.*, 1974; Brown *et al.*, 1975). A more detailed investigation of cholesteryl ester synthesis and its relationship to lipoprotein uptake requires a direct assay of ACAT activity, and we have adapted the method of Goodman *et al.* (1963) for use with preparations of fibroblast membranes. The present paper reports the characteristics of the enzyme and a comparison under a variety of conditions, with substrate cholesterol derived from

Abbreviations used: LDL, low-density lipoprotein; ACAT, acyl-coenzyme A:cholesterol acyltransferase (EC 2.3.1.26).

different sources, between ACAT activity in membrane preparations and the incorporation of oleic acid into cholesteryl esters by intact cells.

The receptor-mediated uptake of LDL by cultured fibroblasts leads to an increase in the synthesis of cholesteryl esters within the cells (Goldstein & Brown, 1977). If the cells are grown in medium supplemented with linoleic acid the uptake and degradation of LDL is increased (Gavigan & Knight, 1981), and we show here that there is a corresponding increase in cholesteryl ester synthesis. In liver microsomes, ACAT activity is increased if the membranes are incubated with a cholesterol-rich donor lipoprotein (Hashimoto & Dayton, 1979). We have been able to confirm this result with fibroblast membranes and to show that the membrane-bound pool of cholesterol that acts as a substrate for ACAT can apparently equilibrate with that of the donor. This property has been exploited to investigate the mechanisms behind the effects of LDL and linoleic acid. ACAT activity was assayed after equilibration of membranes with donor lipoprotein in order to discover whether the increases in ester synthesis were accompanied by an increase in ACAT activity through activation or induction of the enzyme or merely resulted from an increased supply of cholesterol to the substrate pool.

Methods and materials

The methods used for the preparation of LDL (ρ 1.019–1.055 g/ml) and lipoprotein-deficient serum, for the maintenance of human skin fibroblasts, and their preparation for experiments have been described previously (Gavigan & Knight, 1981; Knight & Soutar, 1982).

For the measurement of [^{14}C]oleate incorporation into cholesteryl esters by intact cells, a solution of phosphate-buffered saline [Gibco (Europe)] containing 4% (w/v) bovine serum albumin and 4 mM-[^{14}C]oleate (approx. 3.5 d.p.m./pmol) was added to the culture medium (pH 7.4) to give a final concentration of 0.15 mM-[^{14}C]oleate. After incubation for 60 min at 37°C the cells were harvested and the cholesteryl esters extracted, separated and assayed for radioactivity essentially as described by Goldstein *et al.* (1974).

To prepare membranes from cultured fibroblasts the monolayers were washed three times with ice-cold phosphate-buffered saline and harvested by scraping into 1 ml of the same buffer. Cells from duplicate dishes were pooled and centrifuged at 200g for 5 min at 4°C. The cell pellet was resuspended in 200 μl of 10 mM-potassium phosphate buffer, pH 7.4, containing 2 mM-dithiothreitol and left at 0°C for 10 min for the cells lyse to before homogenizing (30 strokes) in a Potter–Elvehjem

glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 100 000g for 10 min at 4°C in a Beckman 42.2 Ti rotor and the pellet was resuspended in 200 μl of homogenizing buffer.

For the equilibration of cholesterol between membranes and donor lipoprotein, 100 μl of homogenizing buffer containing 50 μg of membrane protein was mixed with 100 μl of phosphate-buffered saline containing 10 mg of protein/ml of LDL from a homozygous familial-hypercholesterolaemic subject. After 60 min at 37°C the membranes were re-isolated by centrifugation at 100 000g in a 42.2 Ti rotor as before, and resuspended in 100 μl of homogenizing buffer.

ACAT activity in membrane suspensions was assayed by a method based on that of Goodman *et al.* (1963). Samples were assayed in duplicate in a final volume of 50 μl containing 100 mM-potassium phosphate buffer, pH 7.4, 2 mM-dithiothreitol, 4 mM-MgCl₂, 12 mg of bovine serum albumin/ml, approx. 15 μg of membrane protein (30 μl of suspension) and 20 μM -[1- ^{14}C]oleoyl-CoA (125 d.p.m./pmol). After incubation at 37°C for 15 min the reaction was stopped by the addition of 1 ml of chloroform/methanol (2:1, v/v). Cholesteryl esters were extracted, separated by t.l.c. and assayed for radioactivity as described by Goldstein *et al.* (1974).

Protein was assayed by the method of Lowry *et al.* (1951), or, in samples containing dithiothreitol, by the method of Bensadoun & Weinstein (1976), with bovine serum albumin as standard.

All constituents of tissue culture media and Dulbecco's phosphate-buffered saline were obtained from Gibco Europe, Paisley, Scotland, U.K., and Lux Contur Petri dishes from Lux Scientific Corp., 1000 Oaks, CA, U.S.A. [1- ^{14}C]Oleic acid (sp. radioactivity >50 Ci/mol) and [1- ^{14}C]oleoyl-CoA (sp. radioactivity 50 Ci/mol) were purchased from Amersham International, Amersham, Bucks., U.K. Palmitic acid, oleic acid, linoleic acid (all approx. 99% pure), mevalonic acid lactone and bovine serum albumin (fraction V) were from Sigma Chemical Co., St. Louis, MO, U.S.A., and cholesterol (>99% pure) was from BDH, Poole, Dorset, U.K.

Results

ACAT activity in fibroblast membranes

All of the ACAT activity in cultured fibroblasts was associated with cellular membranes. In order to study the enzyme in cultured fibroblasts it was necessary to modify the original assay method described by Goodman *et al.* (1963). By increasing the proportion of membranes and reducing the total volume to 50 μl it was possible to assay ACAT activity reliably with membranes prepared from fibroblasts pooled from only two 60 mm culture

Fig. 1. The effects of LDL (a) and cholesterol (b) on ACAT activity

Normal fibroblasts were pre-incubated for 24 h with lipoprotein-deficient serum (2.5 mg of protein/ml) and then for various periods with lipoprotein-deficient serum alone (O) or lipoprotein-deficient serum containing either 100 μg of protein/ml of LDL (●) or 50 μg of cholesterol/ml (■). At each time ACAT activity was assayed in membranes prepared from cells from two duplicate 60 mm dishes and the values shown are the averages of duplicate assays.

dishes. No extra cholesterol was added to the assay mixture. Thus the cholesterol used as substrate by the enzyme was derived entirely from that already present on the membranes. Under all conditions ACAT exhibited an apparent K_m of approx. 8 μM for the second substrate, oleoyl-CoA. Whatever the source of membranes, observed activity was directly proportional to the concentration of membrane protein up to approx. 20 μg of protein/assay and the rate of formation of cholesteryl oleate was constant for at least 15 min.

ACAT activity was low, and remained low, in membranes prepared from fibroblasts that had been incubated for 24 h in medium containing lipoprotein-deficient serum. The addition of LDL to these cells caused a rapid increase in activity, followed by an equally rapid fall to almost the original value (Fig. 1a). The magnitude of the increase depended upon the concentration of LDL added, but with each concentration the greatest activity was observed 7–8 h after the addition of the lipoprotein. There was an apparently saturable relationship between this maximal activity and the concentration of LDL in the incubation medium (Fig. 2a). ACAT activity in fibroblast membranes was also increased if non-esterified cholesterol was added to the culture medium. Activity reached a maximum 24 h after the addition of the cholesterol (Fig. 1b) and remained high for at least a further 24 h. Cholesterol, unlike LDL, could increase ACAT activity in fibroblasts from a homozygous familial-hypercholesterolaemic subject. There was a similar concentration-dependent increase in activity in both types of cell, which was apparently saturated at a concentration of 100 μg of cholesterol/ml (Fig. 2c). The ability to stimulate cholesterol esterification was

Fig. 2. Relationships between ACAT activity and the concentration of LDL (a), mevalonic acid (b) and cholesterol (c) in the incubation medium

Fibroblasts were pre-incubated with lipoprotein-deficient serum for 24 h before the addition of the required concentrations of LDL (●), mevalonic acid (▽) or cholesterol (■, ▲). (a) Membranes were prepared from normal cells 7 h after the addition of LDL. (b) Membranes were prepared from normal cells 24 h after the addition of mevalonic acid. (c) Membranes were prepared from normal (■) cells or cells from a homozygous familial-hypercholesterolaemic subject (▲) 24 h after the addition of cholesterol. ACAT activity in the membrane preparations was assayed as described in the Methods and materials section and the values shown are the averages of duplicate assays.

not limited to cholesterol supplied from outside the cell. Incubation of fibroblasts with mevalonic acid, an intermediate in the pathway of cholesterol

synthesis, also caused a concentration-dependent increase in ACAT activity, of similar magnitude to that produced by LDL (Fig. 2b).

Comparison with cellular oleate incorporation

In order to be able to relate the changes in ACAT activity to changes in net cholesterol esterification in intact cells, incorporation of [14 C]oleate into cholesteryl esters by fibroblasts was measured for a period of 1 h at various times after the addition of LDL or cholesterol. There was essentially no lag before the [14 C]oleate appeared in cholesteryl esters and the incorporation gave an indication of the rate of cholesteryl ester formation at that time. Incorporation of oleate changed in a similar way to ACAT activity in response to LDL or cholesterol. Incubation of fibroblasts with LDL produced a transient increase in the rate of incorporation with a peak after 8–9 h, whereas cholesterol produced a more gradual, but prolonged, effect. As with ACAT, the effect of LDL upon the rate of oleate incorporation into cholesteryl esters was concentration-dependent and saturable (Fig. 3). Fibroblasts from a heterozygous familial-hypercholesterolaemic subject incorporated oleate into cholesteryl esters at approximately one-half of the rate observed with normal cells, whereas fibroblasts from a homozygous subject incorporated very little oleate, even at the highest concentration of LDL (Fig. 3).

Table 1 (the two columns on the left of the Table) shows values of ACAT activity in fibroblast membranes and the rate of oleate incorporation by intact cells, obtained using concentrations of LDL or cholesterol that saturated each process. The similarity between the values for net oleate incorporation and the corresponding values for ACAT,

Fig. 3. Relationship between the concentration of LDL and the incorporation of [14 C]oleate into cholesteryl esters by normal fibroblasts (●) and fibroblasts from heterozygous (□) and homozygous (▲) familial-hypercholesterolaemic subjects

Cells were pre-incubated for 24 h with lipoprotein-deficient serum before the addition of LDL. After 7 h at 37°C the cells were incubated for 1 h with [14 C]oleate and the incorporation into cholesteryl esters was determined as described in the Methods and materials section. Values are averages of duplicate incubations.

Table 1. The effects of LDL and cholesterol on [14 C]oleate incorporation by intact cells and ACAT activity in isolated membranes

Normal fibroblasts were pre-incubated for 24 h with lipoprotein-deficient serum (2.5 mg of protein/ml) and then for the periods shown with the same medium containing 100 μ g of protein/ml of LDL or 50 μ g/ml of cholesterol. At the appropriate times cells were incubated for 1 h with [14 C]oleate to determine [14 C]oleate incorporation or were assayed for membrane ACAT activity before and after equilibration of the membranes with donor lipoprotein (see the Methods and materials section for details). Values shown are means $\pm$ S.E.M. for the numbers of separate incubations shown in parentheses. *Values significantly higher than those of controls incubated with lipoprotein-deficient serum alone ($P < 0.05$; Student's t test). All values obtained after incubation for 24 h with cholesterol were significantly higher ($P < 0.05$; Student's t test) than the corresponding values obtained after incubation for 7 h with LDL.

Incubations	[14 C]Oleic acid incorporated into cholesteryl esters (pmol/min per mg of cell protein)	ACAT activity (pmol/min per mg of membrane protein)	
		Unequilibrated membranes	Equilibrated membranes
Lipoprotein-deficient serum alone	2.5 $\pm$ 0.9 (15)	7.5 $\pm$ 1.2 (10)	15.1 $\pm$ 3.9 (7)
3 h with LDL		12.6 $\pm$ 3.2 (4)	13.8 $\pm$ 2.1 (4)
7 h with LDL	32.9 $\pm$ 8.9 (7)*	28.1 $\pm$ 2.8 (9)*	17.7 $\pm$ 2.6 (7)
16 h with LDL		7.6 $\pm$ 0.6 (2)	16.5 $\pm$ 2.7 (6)
24 h with cholesterol	52.7 $\pm$ 7.5 (3)*	66.6 $\pm$ 7.0 (8)*	30.5 $\pm$ 2.9 (4)*

indicates that the enzyme had not been particularly purified during the preparation of the membranes. The relatively low value for net oleate incorporation in cells incubated with lipoprotein-deficient serum alone presumably resulted from the action of cholesterol esterase in the intact cells. This suggests that the esterase only had a significant effect upon the net incorporation when the rate of cholesterol esterification was low. ACAT activity in fibroblasts incubated for 24 h with a saturating concentration of cholesterol was approximately double the maximum activity observed 7 h after the addition of LDL (Table 1).

Incubation of membranes with cholesterol-rich (donor) lipoprotein

Isolated membranes were incubated with LDL from a homozygous familial-hypercholesterolaemic subject (donor lipoprotein), which was the most cholesterol-rich lipoprotein that was readily available. After incubation the membranes were re-isolated and assayed for ACAT. Fig. 4(a) shows the results obtained with two membrane preparations, one with a high activity before incubation and one with a low activity. ACAT activity in the re-isolated membranes was dependent on the concentration of donor lipoprotein present during the incubation. With a low concentration of donor, activity in both membrane preparations was reduced to a similar low value. As

the concentration of the donor was raised, activity in the re-isolated membranes increased until the concentration reached approx. 4 mg of protein/ml, after which it remained constant. Incubation with a high concentration of donor lipoprotein increased the activity of the less active membrane preparation but still led to a reduction in the activity of the originally more active preparation. These experiments suggested that the cholesterol in the membranes, and particularly that in the substrate pool for ACAT, could exchange and equilibrate with the cholesterol of the donor lipoprotein. Any net transfer was always complete well within the standard 60 min incubation (Fig. 4b). In subsequent experiments, incubations to achieve equilibration of cholesterol always contained 250 μ g of membrane protein/ml with 5 mg of protein/ml of donor lipoprotein.

In order to discover whether the increase in ACAT activity observed when fibroblasts were incubated with LDL or cholesterol could be entirely explained by an increase in the supply of cholesterol to the substrate pool, the membrane preparations were equilibrated with donor lipoprotein (5 mg of protein/ml) and then re-isolated and assayed. The results are shown in the right-hand column of Table 1. The rise and fall in activity produced by LDL that was observed in unequilibrated membranes was not apparent after equilibration. Each preparation then had the same activity, irrespective of the conditions

Fig. 4. ACAT activity after incubation of isolated membranes with donor lipoprotein (LDL from a homozygous familial-hypercholesterolaemic subject)

Membranes were prepared from normal cells that had been pre-incubated for 24 h with lipoprotein-deficient serum (○, △) or for a further 7 h with 100 μ g of protein/ml of LDL (●). For each condition cells were pooled from ten 60 mm dishes. (a) Samples containing 50 μ g of membrane protein were incubated for 60 min at 37°C with the indicated concentration of donor lipoprotein as described in the Methods and materials section. Membranes were re-isolated and assayed for ACAT. (b) Samples containing 50 μ g of membrane protein were incubated for the periods shown with 5 mg of protein/ml of donor lipoprotein. Membranes were re-isolated and assayed for ACAT. The points represent the averages of duplicate assays. Before incubation ACAT activity in membranes from cells incubated with lipoprotein-deficient serum and from cells incubated with LDL was 6.6 and 26.4 pmol/min per mg of protein respectively.

Table 2. *The effect of LDL on ACAT activity in membranes from normal fibroblasts grown with linoleic acid or palmitic acid*

Fibroblasts were grown for 6 days in medium containing 50 μ M-palmitic acid or 50 μ M-linoleic acid and then for 24 h in medium containing lipoprotein-deficient serum and the same fatty acids. Cells were harvested immediately or 7 h after the addition of 100 μ g of protein/ml of LDL. Membranes were prepared and assayed for ACAT activity before and after equilibration of the membranes with donor lipoprotein (see the Methods and materials section for details). Values shown are means $\pm$ s.e.m. of four separate experiments. *Values significantly higher ($P < 0.05$; Student's *t* test) than those of corresponding controls incubated with lipoprotein-deficient serum alone. All values for cells grown with linoleic acid were significantly higher ($P < 0.05$; paired *t* test) than the corresponding values for cells grown with palmitic acid.

Incubation conditions	ACAT activity (pmol/min per mg of protein)	
	Unequilibrated membranes	Equilibrated membranes
Cells grown in palmitate		
Lipoprotein-deficient serum	7.0 $\pm$ 1.7	14.1 $\pm$ 2.1
7 h with LDL	17.2 $\pm$ 3.4*	16.4 $\pm$ 1.8
Cells grown in linoleate		
Lipoprotein-deficient serum	15.8 $\pm$ 3.1	21.0 $\pm$ 0.8
7 h with LDL	36.2 $\pm$ 8.3*	31.9 $\pm$ 2.2*

of culture. In contrast, equilibrated membranes from cells incubated for 24 h with cholesterol exhibited a much greater ACAT activity than equilibrated membranes from cells incubated with lipoprotein-deficient serum alone (Table 1).

Cells enriched with linoleate or palmitate

After incubation of fibroblasts with lipoprotein-deficient serum, ACAT activity in unequilibrated membranes from cells grown with linoleic acid was significantly higher than that of cells grown with palmitic acid (Table 2). LDL produced the normal transient increase in ACAT activity, which, in each case, was greatest 7 h after the addition. This maximal activity was much greater in the cells that had been grown in linoleic acid (Table 2). Furthermore, the increase in activity produced by LDL in these cells was twice that produced by LDL in the cells grown in palmitic acid. After equilibration with donor lipoprotein, membranes from cells grown with linoleic acid still exhibited a greater ACAT activity than membranes from cells grown with palmitic acid, both in the presence and in the absence of LDL (Table 2). As with cells maintained in normal,

unsupplemented medium, ACAT activity in equilibrated membranes from cells grown in the presence of palmitic acid did not increase when the cells were incubated with LDL. However, with cells grown in linoleic acid, ACAT activity in fully equilibrated membranes was markedly increased during incubation of the intact cells with LDL (Table 2).

Discussion

When normal fibroblasts are incubated without lipoproteins they express specific surface receptors that enable them to bind, internalize and degrade LDL and so release non-esterified cholesterol within the cell. Expression of receptors is tightly controlled, and as the cellular cholesterol content increases the number of surface receptors is reduced (Goldstein & Brown, 1977). The rapid increase in both oleate incorporation into cholesteryl esters and membrane ACAT activity in such cells after the addition of LDL reflected the initially high rate of uptake and degradation of the lipoprotein. As the number of surface binding sites, and thus the supply of unesterified cholesterol, was reduced, the rate of formation of cholesteryl esters reached a peak and then fell. Cells from homozygous familial-hypercholesterolaemic subjects, which cannot express functional receptors due to a genetic defect, did not show any increase in the rate of cholesteryl ester formation on incubation with LDL. Conversely, cells that had been grown in the presence of linoleic acid, which degrade LDL at a greater rate than those grown in normal medium or with palmitic acid (Gavigan & Knight, 1981) exhibited a correspondingly greater ACAT activity in their isolated membranes. It was not only receptor-mediated uptake of LDL that could increase ACAT activity. The addition of mevalonic acid to stimulate endogenous cholesterol synthesis had a similar effect, and the uptake of non-esterified cholesterol from the medium, which was not subject to any metabolic control, could increase ACAT activity to a value that was approximately twice as high as the maximum observed with LDL.

These results suggest that the rate at which cholesterol is deposited within the cells is an important factor in regulating the activity of ACAT on the membranes. However, given an increase in the supply of cholesterol, there are a number of ways in which an increase in activity could be achieved. The cholesterol used as substrate by ACAT, like the enzyme itself, is membrane-bound (Balasubramaniam *et al.*, 1978b). Since it is a small proportion of the total membrane cholesterol (Balasubramaniam *et al.*, 1978a), once the membranes have been isolated there is only a limited amount of substrate available to the enzyme. For this reason great care was taken to ensure that we were

measuring the initial rate of reaction, whatever the source of the membrane preparation. Under these circumstances the activity observed was determined by the amount of enzyme present in the preparation, its degree of activation and the amount of cholesterol in the substrate pool. Incubation of the membranes with donor lipoprotein resulted in an apparent equilibration of cholesterol between the donor and the membranes, including the substrate pool, and provided an opportunity to gain some insight into the mechanisms by which ACAT activity was modulated. There was a saturable relationship between the activity observed after equilibration and the concentration of the donor lipoprotein. Although incubation with a saturating concentration of donor did not elicit the maximum possible ACAT activity, it provided conditions in which membrane preparations that differed before incubation only in the size of the substrate pool, would give the same activity when subsequently assayed. This was the case with membranes from cells that had been grown in normal medium, preincubated with lipoprotein-deficient serum and then incubated with LDL. After each had been equilibrated with donor, membranes prepared when esterification was at its peak had the same activity as those prepared before the addition of LDL or when the rate of esterification had subsided. Thus the differences in ACAT activity in unequilibrated membranes could be explained entirely by differences in the size of the substrate pool. The results support the proposal that the variations in ACAT activity after incubation of fibroblasts with LDL merely reflect the variations in the supply of substrate for the enzyme from the degradation of the lipoprotein. Doolittle & Chang (1982) have recently reported experiments with ACAT reconstituted into artificial membranes that also indicate that the effects of LDL are not associated with changes in the amount of enzyme present. The response of fibroblasts to a prolonged increase in cholesterol uptake was different from the response to the transient increase brought about by LDL. Membranes from cells that had been incubated for 24 h in medium containing non-esterified cholesterol had a greater ACAT activity after equilibration than those from cells left in lipoprotein-deficient serum, suggesting that, in addition to any increase in substrate supply, there had been changes in the composition of the membranes that produced a change in enzyme activity or a different distribution of membrane cholesterol during equilibration with donor.

With fibroblasts grown in medium supplemented with linoleic acid, even the effects of LDL could not be entirely explained by an increase in the size of the substrate pool. ACAT activity in these cells, both before and after the addition of LDL, was greater than that in cells grown with palmitic acid. These

differences were still apparent after the membranes had been equilibrated with donor. Indeed, equilibrated membranes from fibroblasts grown with linoleic acid, unlike those from cells grown with palmitic acid or in normal, unsupplemented medium, exhibit a further increase in ACAT activity when the cells were incubated with LDL. To account for these results there must have been at least two separate alterations in membrane composition. Although we cannot deduce the exact nature of these with any certainty, the best interpretation of the available evidence is that an enrichment of cellular phospholipids with linoleic acid led to a change in the structure of the membranes so that there was an increase in the size of the substrate pool after equilibration with donor. Furthermore, when faced with an exceptional increase in the supply of cholesterol, whether from the prolonged uptake of non-esterified cholesterol or from the accelerated degradation of LDL in cells grown with linoleic acid, the fibroblasts were able to increase their potential catalytic activity so that surplus cholesterol was esterified and did not accumulate and interfere with normal membrane function. This increase in catalytic activity could involve an induction of ACAT synthesis or an activation of existing enzyme through a change in membrane fluidity (Spector *et al.*, 1980; Field & Salome, 1982; Suckling *et al.*, 1982) or through a covalent modification such as phosphorylation (Gavey *et al.*, 1982).

Whatever the actual mechanisms involved, the results reported in the present paper indicate that the enhanced rate of degradation of LDL shown by fibroblasts that have been grown with linoleic acid leads to a corresponding increase in the rate of cholesterol esterification by the cells, accommodated by an increase in the potential ACAT activity of their membranes. In fibroblasts cultured under normal conditions the potential ACAT activity in cells pre-incubated with lipoprotein-deficient serum is adequate to esterify the cholesterol released after the addition of LDL without the need for any further synthesis or activation of the enzyme.

We thank Mr. Dilip Patel for his excellent technical assistance, Dr. S. Balasubramaniam for his advice during the early stages of this work and Mrs. Jean De Luca for her secretarial help.

References

- Balasubramaniam, S., Mitropoulos, K. A. & Venkatesan, S. (1978a) *Eur. J. Biochem.* **90**, 377–383
- Balasubramaniam, S., Venkatesan, S., Mitropoulos, K. A. & Peters, T. J. (1978b) *Biochem. J.* **174**, 863–872
- Bensadoun, A. & Weinstein, D. (1976) *Anal. Biochem.* **70**, 241–250

- Brown, M. S., Faust, J. R. & Goldstein, J. L. (1975) *J. Clin. Invest.* **55**, 783-793
- Doolittle, G. M. & Chang, T.-Y. (1982) *Biochim. Biophys. Acta* **713**, 529-537
- Field, F. J. & Salome, R. G. (1982) *Biochim. Biophys. Acta* **712**, 557-570
- Gavey, K. L., Trujillo, D. L. & Scallen, T. J. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 1399
- Gavigan, S. J. P. & Knight, B. L. (1981) *Biochim. Biophys. Acta* **665**, 632-635
- Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46**, 897-930
- Goldstein, J. L., Dana, S. E. & Brown, M. S. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4288-4292
- Goodman, D. S., Deykin, D. & Shiraturi, T. (1963) *J. Biol. Chem.* **239**, 1335-1345
- Hashimoto, S. & Dayton, S. (1979) *Biochim. Biophys. Acta* **573**, 354-360
- Knight, B. L. & Soutar, A. K. (1982) *Biochem. J.* **202**, 145-152
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Ross, R. (1981) *Arteriosclerosis* **1**, 293-311
- Spector, A. A., Kaduce, T. L. & Dane, R. W. (1980) *J. Lipid Res.* **21**, 169-179
- Suckling, K. E., Boyd, G. S. & Smellie, C. G. (1982) *Biochim. Biophys. Acta* **710**, 154-163